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Abstract: Clogging in HSSF CW may result in a reduction of system's life-span or treatment efficiency. Current available techniques to assess the degree of clogging in HSSF CW are time consuming and cannot be applied on a continuous basis. Main objective of this work was to assess the potential applicability of microbial fuel cells for continuous clogging assessment in HSSF CW. To this aim, two replicates of a membrane-less microbial fuel cell (MFC) were built up and operated under laboratory conditions for five weeks. The MFC anode was gravel-based to simulate the filter media of HSSF CW. MFC were weekly loaded with sludge that had been accumulating for several years in a pilot HSSF CW treating domestic wastewater. Sludge loading ranged from ca. 20 kg TS.-1.m-3.year at the beginning of the study period up to ca. 250 kg TS.-1.m-3.year at the end of the study period. Sludge loading applied resulted in sludge accumulated within the MFC equivalent to a clogging degree ranging from 0.2 years (ca. 0.5 kg TS/m3wetland) to ca. 5 years (ca. 10 kg TS/m3wetland). Results showed that the electric charge was negatively correlated to the amount of sludge accumulated (degree of clogging). Electron transference (expressed as electric charge) almost ceased when accumulated sludge within the MFC was equivalent to ca. 5 years of clogging (ca. 10 kg TS/m3wetland). This result suggests that, although longer study periods under more realistic conditions shall be further performed, HSSF CW operated as a MFC has great potential for clogging assessment.

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Opposed Reviewers:

Dear Editor,

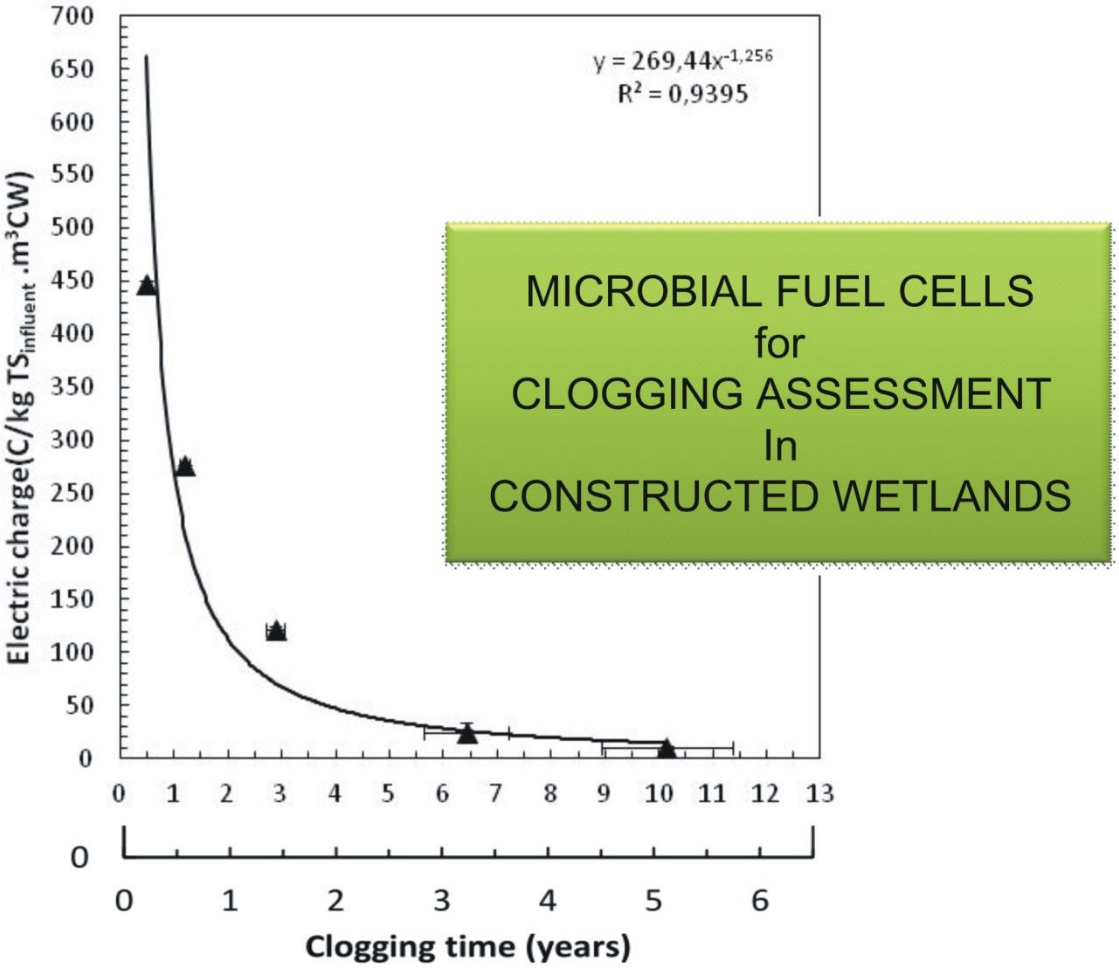
Please find enclosed the research paper entitled ***"Microbial fuel cells for clogging assessment in constructed wetlands"*** for its potential publication in Science of the Total Environment. The paper is based on original experimental data. Briefly, the paper addresses the potential use of microbial fuel cells for clogging assessment in constructed wetlands. To the knowledge of the authors this is the first time this topic is addressed in current literature.

Thank you in advance for your attention.

Best regards,

Jaume Puigagut

*Graphical Abstract



HIGHLIGHTS

-Microbial fuell cells are used as tool for clogging assessment in constructed wetlands

-Microbial fuell cells were loaded with sludge from construced wetlands

-Sludge retained within the systems simulated a clogging time ranging from 0.2 to ca. 5 years

-Electrons transferred decreased potentially as function of sludge loading

-Microbial fuel cells has potential for clogging assessment tool

Microbial fuel cells for clogging assessment in constructed wetlands

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Abstract.

Clogging in HSSF CW may result in a reduction of system's life-span or treatment efficiency. Current available techniques to assess the degree of clogging in HSSF CW are time consuming and cannot be applied on a continuous basis. Main objective of this work was to assess the potential applicability of microbial fuel cells for continuous clogging assessment in HSSF CW. To this aim, two replicates of a membrane-less microbial fuel cell (MFC) were built up and operated under laboratory conditions for five weeks. The MFC anode was gravel-based to simulate the filter media of HSSF CW. MFC were weekly loaded with sludge that had been accumulating for several years in a pilot HSSF CW treating domestic wastewater. Sludge loading ranged from ca. 20 kg $TS^{-1}.m^{-3}$.year at the beginning of the study period up to ca. 250 kg $TS^{-1}.m^{-3}$.year at the end of the study period. Sludge loading applied resulted in sludge accumulated within the MFC equivalent to a clogging degree ranging from 0.2 years (ca. 0.5 kg $TS/m^3_{wetland}$) to ca. 5 years (ca. 10 kg $TS/m^3_{wetland}$). Results showed that the electric charge was negatively correlated to the amount of sludge accumulated (degree of clogging). Electron transference (expressed as electric charge) almost ceased when accumulated sludge within the MFC was equivalent to ca. 5 years of clogging (ca. 10 kg $TS/m^3_{wetland}$). This result suggests that, although longer study periods under more realistic conditions shall be further performed, HSSF CW operated as a MFC has great potential for clogging assessment.

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Introduction

Horizontal subsurface flow constructed wetlands (HSSF CW) are natural wastewater treatment systems that consist of gravel beds planted with macrophytes (García et al., 2010). In HSSF CWs pollutants are removed by means of physical, chemical and biological processes. HSSF CWs are generally used as secondary treatment units for the removal of contaminants (mainly organic matter) contained in domestic wastewater. HSSF CWs suffers from a progressive media obstruction, the so-called clogging process. Clogging is a complex phenomena derived from the retention of organic/inorganic particles, precipitation, biofilm formation and root-system growth (Knowles et al., 2011; Pedescoll et al., 2011a). It is widely accepted that clogging is one of the major operational problems of HSSF CWs (Kadlec and Wallace, 2009; Knowles et al., 2011; Nivala et al., 2012). Loss of hydraulic conductivity, creation of preferential water-flows, ponding and reduction of both treatment efficiency and system's life-span are some of the consequences of clogging (Caselles-Osorio and García, 2006; Knowles et al., 2011; Nivala et al., 2012; Wallace and Knight, 2006). To mitigate/reverse the clogging, different strategies (both preventive and/or restorative) are currently envisaged (Nivala et al., 2012). The quantification of total solids entrapped within the filter media is one of the most widely used techniques/strategies to assess the degree of clogging of HSSF CW. However, this procedure is time consuming, results in methodological inaccuracies due to sample manipulation and cannot be applied on a continuous basis. A great extent of the particulate material retained within HSSF CW is of organic nature (Knowles et al., 2011; Pedescoll et al., 2011a).Therefore, microbial fuel cells (MFC) could be a suitable technology for clogging assessment. MFCs are bioelectrochemical devices that generate electricity from the degradation of organic matter by means of exoelectrogenic bacteria as catalysts (Logan, 2008). MFC implementation in HSSF CWs has been already carried out taking benefit of the natural redox gradient existing between the upper and the bottom part of the treatment bed (Corbella et al., 2016, 2015, 2014). Furthermore, MFCs have been also described as a suitable technology for bioindication purposes. Accordingly, MFC can produce an electric current proportional to the amount of organic matter present in the system. So far, the electric current generated by a MFC has been successfully correlated to organic substrates such as artificial wastewater and real domestic wastewater (Chang et al., 2004; Di Lorenzo et al., 2009; Gil et al., 2003; Juang et al., 2011; Liu et al., 2000). The main objective of the present work was to determine whether MFC could be of use to quantitatively assess the total amount of organic particles retained within the gravel matrix so as to have a non disruptive, continuous clogging assessment tool. To the authors knowledge this is the first attempt to use MFC as a clogging assessment tool.

Materials and methods

Set-up configuration

For the purpose of this work two lab-scale membrane-less microbial fuel cells (MFC) were set up and operated for 5 weeks (Figure 1). Each MFC consisted of two chambers (the cathodic and anodic chambers). The anodic chamber consisted of a PVC cylinder of 9 cm diameter and 30 cm of height filled up with gravel wrapped in stainless steel mesh (marine grade 316L). The anode camber of the MFC was designed to simulate a core of a shallow wetland gravel bed where the electrons derived from exoelectrogens organic matter oxidation would be transferred to the electron collector (stainless steel mesh). In order to ensure adequate mixing conditions, water inside the anode chamber was continuously recirculated by means of a peristaltic pump (Damova MP-3035-6M; Toshiba VF-nC3). The cathode chamber consisted of a PVC cylinder placed just above the anode chamber filled up with 5 pieces of graphite felt (Alfa Aesar, 1.12 cm thick, 99.9 %; metal basis) of 60 cm^2 each inter-connected using stainless steel wires (marine grade 316L). A layer of glass wool was placed between the anode and the cathode chamber so as to avoid any oxygen leaking from the cathode (Venkata Mohan et al., 2008). The anode and the cathode were externally connected by means of copper wires through an external resistance of 1000 ohms. MFCs were operated under MFC mode thus no external energy was provided. Finally, voltages generated were measured across the external resistance and stored every minute by means of a datalogger (Campbell Scientific CR1000).

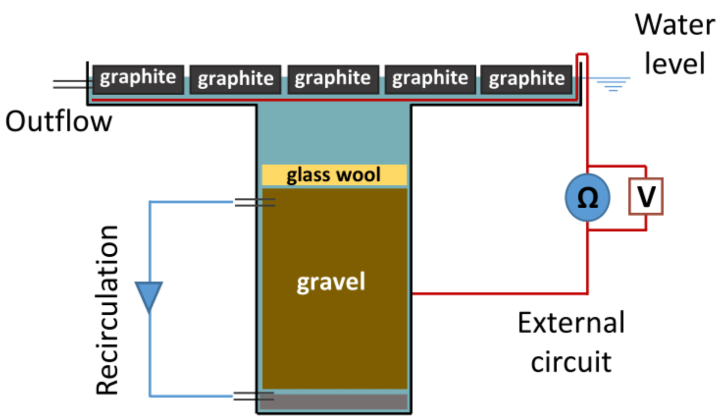

Figure 1 Out-line of the MFC used to conduct the experiment.

Sludge collection, experimental design and clogging assumptions

Sludge was supplied to the MFC on a weekly basis and current produced by the MFC was monitored for six days after each sludge load. MFCs were loaded in batch mode with sludge collected from the bottom of a HSSF CW pilot plant that had been treating domestic wastewater for several years (Corbella et al., 2016). Solids accumulation in HSSF CWs is one of the main causes for clogging (Caselles-Osorio et al., 2007). Therefore, the introduction of HSSF CW sludge was considered to fairly simulate the clogging process derived from solids accumulation within the filter media of HSSF CW. Note that although particles accumulation is one of the main processes contributing to the progressive media obstruction in wetlands, clogging is also the result of other processes such as precipitation, root growth, biofilm growth, etc…. Therefore, we shall point out that in this work we are mainly addressing the clogging process derived from particles accumulation. The experiment lasted until the electric charge produced by the MFC was close to zero (five weeks). The sludge used to load the systems was collected and concentrated by sedimentation during 1 week at 4ºC. The concentrated sludge was then analyzed (CW sludge, Table 1) and lately diluted with tap water to achieve the desired concentrations. Each week the systems were fed with an increasing sludge load. Accordingly, sludge loading ranged from ca. 20g $TS^{-1}.m^{-3}$.year at the beginning of the study period up to ca. 250 g $TS^{-1}.m^{-3}$.year at the end of the study period (Table 1). For the estimation of clogging time essayed we assumed that one year of clogging corresponded to the accumulation of 2 kg TS/m^3.year (Caselles-Osorio et al., 2007). The sludge provided to each of the MFC replicate was as homogeneously distributed as possible along the length of the anode. It is important to note that no wastewater was used in this experiment (sludge provided was mixed with tap water prior to supply it to the MFC). Therefore, the electric signal recorded in our MFC derived solely from the oxidation of the sludge provided.

Cumulative sludge retention (ACC in kg TS/m^3) at a certain week (w) was calculated by means of the sum of the sludge accumulated during previous weeks (ACC_i in kg TS/m^3) plus the sludge loading at the week in course ($\mathrm{TS(IN)_w}$ in kg TS/m^3)(Eq.1). The sludge accumulated (ACC_i) at a specific week (i) was calculated as the sludge supplied ($TS\ (IN)_i$ in kg TS/m^3) minus the sludge at the effluent ($TS(OUT)_i$ in kg TS/m^3) (Eq. 2).

$$\textbf{Eq. 1}\ \mathrm{C_w} = \sum_{i=1}^{i=w-1} \mathrm{ACC_i} + \mathrm{TS(IN)_w} \qquad \textbf{Eq. 2}\ \mathrm{ACC_i} = \mathrm{TS(IN)_i} - \mathrm{TS(OUT)_i}$$

Table 1 Average and standard deviation (in brackets) of physical and chemical parameters of the sludge employed and experimental conditions tested.

		Sludge					Operational conditions			
		TS *(g/L)*	**VS** *(g/L)*	**DQO_{tot}** *(mgO_2/L)*	**DQO_{sol}** *(mgO_2/L)*	**NH_4^+ - N** *($mgNH_4^+$-N/L)*	**Sludge Loading** *($kgTS/m^3CW \cdot year$)*	**Organic Loading** *($gO_2/m^3CW \cdot year$)*	**Sludge accumulated** *($kgTS/m^3CW$)*	**Cumulative clogging time** *(years)*
Raw		39.1 (0.7)	25.5 (1.3)	58,100 (1,926)	933 (109)	87.4 (1.3)	-	-	-	-
Experimental Weeks	1	1.0	0.6	741	87	2.8	21.5	15.5	0.5 (0.0)	0.2 (0.0)
	2	1.8	1.0	918	144	8.9	37.6	19.1	1.2 (0.1)	0.6 (0.1)
	3	4.0	2.5	1,640	249	10.7	82.5	34.2	2.9 (0.2)	1.4 (0.1)
	4	8.7	6.2	5,408	524	17.9	180.9	112.8	6.4 (0.8)	3.2 (0.4)
	5	12.1	8.5	16,700	674	24.6	252.0	348.3	10.2 (1.2)	5.1 (0.6)

Physico-chemical analyses

Samples were analyzed before and after each batch test (Table 1). Quality parameters analyzed during the experiment were that of total solids (TS), volatile solids (VS), total and soluble chemical oxygen demand (COD_{tot} and COD_{sol}) and ammonium nitrogen. Water quality parameters were analyzed following APHA (2005).

Results and discussion

MFC for clogging assessment

Clogging is one of the main operational problems of HSSF CWs and it has been traditionally assessed by means of the quantification of solids accumulated within the gravel matrix (Caselles-Osorio et al., 2007). However, this procedure is time consuming, cannot be applied on a continuous basis and results are not entirely reliable due to methodological inaccuracies derived from sample manipulation (Pedescoll et al., 2011a). To avoid direct measurements, two indirect clogging assessment methodologies have been proposed: (1) hydraulic conductivity measurements (Pedescoll et al., 2012, 2011b), and (2) tracer tests (Nivala et al., 2012). However, these methods still require of exhaustive and time consuming sampling/measuring campaigns and cannot be applied on a continuous basis. In this study MFCs were loaded with increasing sludge dosages. As expected, the sludge retained within the systems had a direct effect on the MFC functioning (Figure 2). The electric current generated by a MFC has been positively correlated to the concentration of organic substrates such as artificial wastewater and real domestic wastewater (Chang et al., 2004; Di Lorenzo et al., 2009; Gil et al., 2003; Juang et al., 2011; Liu et al., 2000). However, our results showed that sludge retention was negatively correlated with the electrons transferred to the circuit for each batch test performed (Figure 2). Hence, the higher the sludge accumulated within the MFC the lower the amount of electrons transferred. These results are consistent with previous publications, where higher organic matter concentrations were negatively correlated to the current generated in a single chamber MFC (Sharma and Li, 2010). Author's hypothesis behind the reduction of current generated under higher particles accumulation is that of the bacterial activity decreases due to limited substrate availability. Accordingly, the lower the pore space available within the filter media, the lower the substrate availability for exoelectrogens and the lower the amount of electrons transferred. This hypothesis is consistent with that described for the general functioning of bacterial activity in horizontal subsurface flow constructed wetlands (Samsó and García, 2014).

Correlations between specific electric charge and solids accumulated followed a potential equation, regardless the contact time considered (Figure 2). Results showed that after an equivalent clogging time of ca. 5 years the MFC was no longer able to produce any significant electric charge (Figure 2). This result suggests that, although longer study periods under more realistic conditions shall be further performed, MFC has great potential to be used as an indirect, continuous clogging assessment tool in constructed wetlands.

It is important to point out that the specific electric charge (given a certain amount of sludge accumulated within the MFC) increased with the contact time (especially between 0.2 and 1.4 years of clogging) (Figure 3). Accordingly, the higher the elapsed time for a given amount of sludge accumulated the higher the electric charge produced. The production of a higher electric charge after a higher elapsed time is consistent with the fact that hydrolytic bacteria shall cut up complex substrates (such as the sludge here employed) before the exoelectrogens can oxidize simple substrates (such as volatile fatty acids) and transfer the electrons to the anode (Cusick et al., 2010; Kiely et al., 2011). Overall, although the electric charge was dependant on the contact time, the level of correlation between the electric charge and the amount of sludge retained (or clogging time essayed) was of similar magnitude (always showing $R^2>0.9$), regardless the contact time considered (Figure 2). Therefore, from our results it seems that there is no specific contact time out of those here considered that results in better MFC performance in terms of clogging assessment.

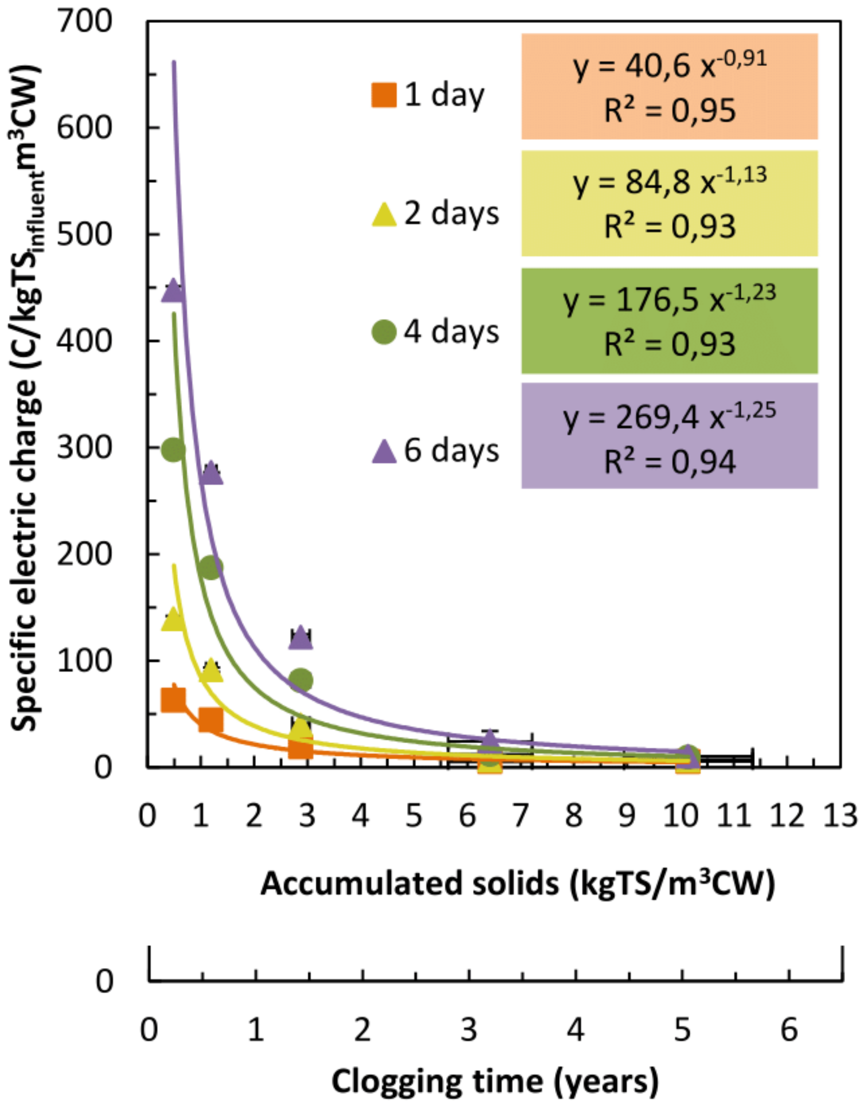

Figure 2. Specific electric charge against both the accumulated total solids (TS) and the equivalent clogging time for a selected set of days of contact time.

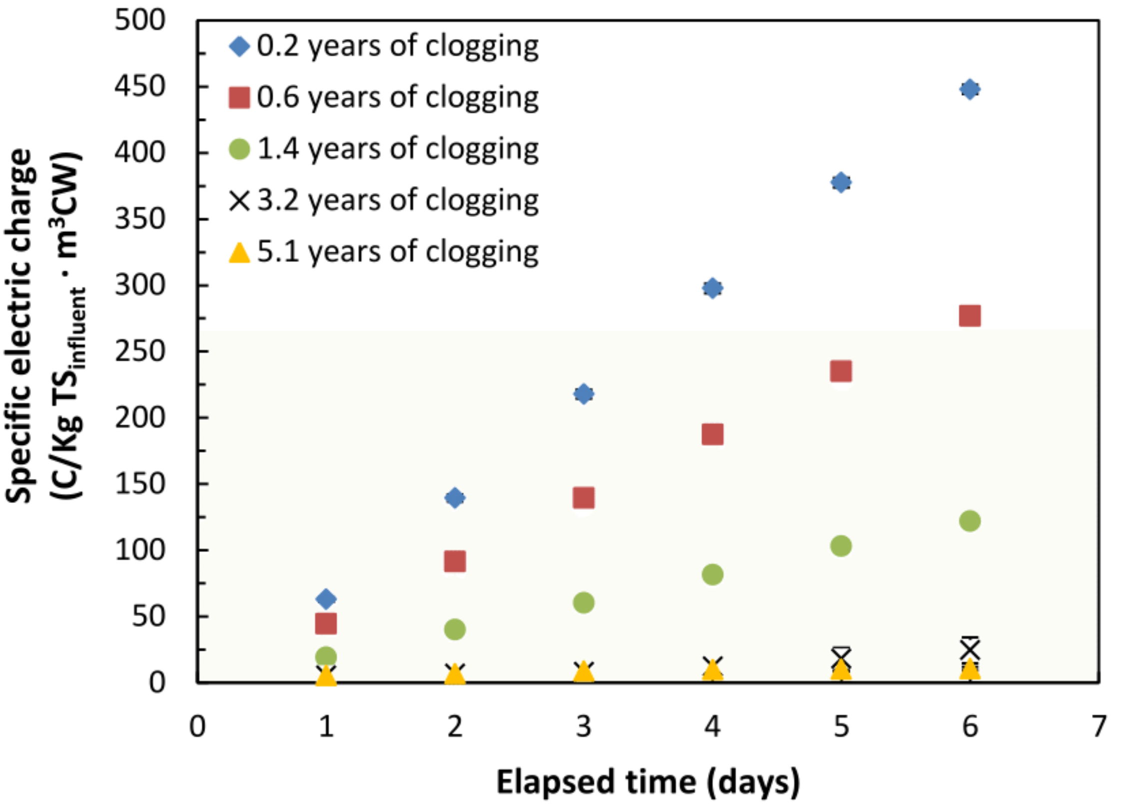

Figure 3. Specific electric charge as function of the elapsed time for a given estimated clogging time. Note that error bars are depicted within the figure, yet due to low data variability it is difficult to appreciate it.

Conclusions

The sludge retained within the microbial fuel cells (MFC) had a direct effect on the MFC performance. Accordingly, the higher the sludge accumulated within the MFC the lower the amount of electrons transferred. The electric charge decreased as function of sludge retained following a potential equation.

After ca. 5 years of estimated clogging time the MFC was no longer able to produce any significant electric charge. This result suggests that, although longer study periods under more realistic conditions shall be further performed, MFC has great potential to be used as an indirect, continuous clogging assessment tool in constructed wetlands.

Electric charge was dependant on the contact time. Accordingly, the higher the contact time the higher the amount of electrons transferred to the circuit. However, the correlation between the electric charge and the amount of sludge retained (or clogging time essayed) was of similar magnitude (always showing R^2>0.9), regardless the contact time considered. Therefore, the accuracy of the MFC for clogging assessment was independent of the contact time considered.

Acknowledgments

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